Reference: *Biol. Bull.*, **159**: 148–161. (August, 1980)

# THE EFFECTS OF TEMPERATURE AND SALINITY ON THE OSMOTIC COMPOSITION OF THE SOUTHERN OYSTER DRILL, *THAIS HAEMASTOMA*

JANE E. HILDRETH[1] AND WILLIAM B. STICKLE

*Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana, 70803*

Temperature and salinity are two of the most important physical factors affecting estuarine organisms. In most studies on the effects of physical variables on estuarine organisms, all factors but one have been rigorously controlled and kept constant. Kinne (1971) argues that multifactorial experiments measuring the effects of two or more environmental factors acting in concert are necessary to understand the functioning of estuarine organisms under natural conditions.

Most previous work on the effects of temperature and salinity on osmoregulation of marine and estuarine invertebrates has been done using osmoregulating crustaceans (Verwey, 1957; Lockwood, 1960; Dehnel, 1962; Todd, 1963; Dehnel and Carefoot, 1965; Taylor *et al.*, 1977). These workers and others have found that most euryhaline invertebrates studied exhibit greater osmoregulatory capabilities in the lower portions of their normal temperature ranges (see Kinne, 1970, 1971, for review).

Many workers have investigated the effects of salinity on the blood solute concentrations of various molluscs, but the possible interaction of salinity and temperature on osmotic and ionic regulation in molluscs has not been studied thoroughly. The present study had two objectives: 1) to investigate the interaction of temperature and salinity on the osmotic composition of an osmoconforming marine gastropod *Thais haemastoma* and 2) to investigate the effects of long-term salinity fluctuations on this snail. Hemolymph osmolality and concentrations of $Na^+$, $K^+$, $Mg^{2+}$, and $Cl^-$ under constant and fluctuating salinity were measured at 10°, 20°, and 30°C. In addition, percent tissue water and the level of ninhydrin positive substances (NPS) in the foot tissue were determined.

## MATERIALS AND METHODS

Specimens of *Thais haemastoma* were collected during September and October, 1977, from Barataria Bay in the vicinity of Grande Isle, La., U.S.A. Ambient salinity at the time of collection varied from 24 to 28‰, water temperature between 26° and 30°C. Snails were returned to the laboratory and placed in aquaria containing Instant Ocean artificial seawater (Eastlake, Ohio) at room temperature and the same salinity as the collection site. Snails were brought to the initial salinity of each experiment by adjusting the salinity (S) 2‰ per day until the desired salinity was reached. The aquaria were then placed in a water bath (20° and 30°C experiments) or a cold room (10°C experiment) and held at this temperature-salinity combination for 14 days before simulated tidal cycles were begun. The oyster drills were held under constant illumination during the acclimation period and all experiments. Live oysters were available as food for the drills at all times.

---

[1] Present Address: Department of Zoology, Oregon State University, Corvallis, Oregon 97331.

### *Constant salinity*

Snails were acclimated to 7.5, 10, 20, and 30‰ S for 5 weeks at 10° and 20°C and 4 weeks at 30°C. At the time of sampling snails were removed from acclimation aquaria and an ambient seawater sample taken. The shell was cracked near the heart and 50–300 μl of hemolymph collected in microcapillary tubes and placed in 400 μl plastic snap-cap centrifuge tubes. Hemolymph samples were centrifuged at 9000 × *g* for 5 min to pellet any particulate matter. The osmolality of hemolymph and seawater was determined with a WESCOR Vapor Pressure Osmometer. Hemolymph and water samples were diluted with deionized water for ion analyses. The foot was removed, blotted, and the weight determined; it was frozen on dry ice and lyophilized to constant weight for the determination of percent tissue water.

Chloride ion concentrations were determined with an Aminco Chloridometer, $Na^+$ and $K^+$ with a Coleman Flame Photometer, and $Mg^{2+}$ with a Perkin-Elmer Model 303 Atomic Absorption Spectrophotometer.

Ninhydrin positive substances (NPS) were determined in the foot tissue. The freeze dried foot was ground to a powder in a Wiley grinding mill and 10 mg tissue incubated 48 hr in 10 mls of 10% 5-sulfosalicylic acid to precipitate proteins and allow complete leaching of the NPS from the tissue. The samples were centrifuged at 12,100 × *g* for 15 min and NPS determined on a 0.1 ml aliquot of the supernatant by the method of Rosen (1957). Leucine was used as the standard. NPS levels are reported as μM NPS/g dry weight and as μM NPS/g tissue water.

### *Salinity fluctuation experiments*

Salinity fluctuations simulated diurnal tidal cycles of 24 hr. A dilution apparatus described by Stickle and Ahokas (1974) was used to simulate tidal cycles (salinity regimes) of 30–10–30‰ and 10–30–10‰ S. The salinity was raised or lowered during the first 10 hr of the cycle, held at this salinity for a 2-hr slack-water period, then returned to the initial salinity during the next 10 hr. After a second 2-hr slack water period the cycle was repeated daily for a total of 21 days. Both cycles were run at 10°, 20°, and 30°C.

Snails were killed and hemolymph samples taken on Days 1, 3, 6, 9, 12, 15, 18, and 21 in the 10°C experiment. Samples were taken on the same days in the other two experiments with the following exceptions: 1) only snails under the 30–10–30‰ salinity regime were sampled on Days 6 and 15 of the 30°C experiment and 2) no samples were taken on Day 9 of the 20°C experiment. These sample periods were omitted to reduce the numbers of animals used in the experiments.

### *Statistical analysis: Constant salinity*

Because the salinity of aquaria varied by as much as 2‰ among the three temperatures tested, data were standardized by expressing osmolality and ion concentrations as the hemolymph concentration minus the seawater concentration. In this way observed differences are due to temperature effects, not to minor variations in salinity among the three experiments.

An analysis of variance (ANOVA) was run to test the effects of temperature, salinity, and temperature-salinity interaction (a 3 × 4 factorial design) on present tissue water, hemolymph osmolality, $Cl^-$, $Na^+$, $Mg^{2+}$, and $K^+$ concentrations, μM NPS/g dry weight, and μM NPS/g tissue water of acclimated snails. Duncan's

TABLE I

*Mean hemolymph (HL)-minus-seawater osmolality and ion concentrations of* Thais haemastoma *held at 12 temperature-salinity combinations (mean percent tissue water in the foot is also given for each temperature–salinity combination). Values given are mean ± standard error (n). Columns or rows joined by the same bar are not significantly different. An * indicates hemolymph-minus-seawater osmolality is significantly different from 0 at the 5% level.*

| | | 7.5‰ | 10‰ | 20‰ | 30‰ |
|---|---|---|---|---|---|
| Osmolality (mOsm) | | | | | |
| Water | | 219 | 292 | 584 | 876 |
| HL | 10°C | 26 ± 17 (4) | 38 ± 13 (5)* | 135 ± 16 (5)* | 23 ± 1 (4)* |
| | 20°C | 5 ± 5 (5) | 27 ± 2 (5)* | 9 ± 1 (5)* | 1 ± 2 (5) |
| | 30°C | 8 ± 5 (5)* | 4 ± 2 (4)* | 29 ± 10 (5) | −34 ± 12 (4) |
| Tissue Water (%) | | | | | |
| Water | | | | | |
| HL | 10°C | 78 ± 1 (6) | 78 ± 2 (5) | 73 ± 2 (4) | 74 ± 1 (2) |
| | 20°C | 77 ± 1 (5) | 76 ± 0 (5) | 76 ± 0 (4) | 76 ± 1 (4) |
| | 30°C | 77 ± 0 (5) | 77 ± 1 (4) | 75 ± 1 (5) | 72 ± 1 (4) |
| $Cl^-$ (mM) | | | | | |
| Water | | 117 | 156 | 312 | 469 |
| HL | 10°C | −6 ± 17 (4) | −20 ± 13 (4) | 74 ± 22 (5) | −17 ± 8 (2) |
| | 20°C | −24 ± 4 (5) | −42 ± 4 (4)* | −22 ± 7 (5) | 8 ± 11 (5) |
| | 30°C | −20 ± 3 (5) | −15 ± 3 (4) | 3 ± 15 (5) | −90 ± 2 (3)* |
| $Na^+$ (mM) | | | | | |
| Water | | 98 | 130 | 261 | 391 |
| HL | 10°C | 52 ± 15 (5) | 29 ± 28 (4) | 20 ± 5 (5) | −7 ± 51 (2) |
| | 20°C | −14 ± 9 (5) | −14 ± 2 (4)* | 40 ± 3 (5) | −37 ± 11 (5) |
| | 30°C | 10 ± 16 (5) | 4 ± 6 (4) | 2 ± 7 (5) | 4 ± 11 (3) |
| $Mg^{2+}$ (mM) | | | | | |
| Water | | 11.4 | 15.2 | 30.4 | 46.6 |
| HL | 10°C | 14.9 ± 2.0 (5)* | 10.9 ± 3.0 (4)* | −11.5 ± 1.6 (5)* | −1.0 ± 2.0 (2) |
| | 20°C | 10.4 ± 1.0 (5)* | 8.7 ± 0.8 (4)* | 8.6 ± 0.8 (5)* | 2.7 ± 2.9 (5) |
| | 30°C | 1.0 ± 2.0 (5) | 3.2 ± 0.7 (4) | 0.2 ± 0.7 (5) | −2.4 ± 2.5 (3) |
| $K^+$ (mM) | | | | | |
| Water | | 2.2 | 2.8 | 5.6 | 8.5 |
| HL | 10°C | 3.5 ± 0.7 (5) | 3.7 ± 1.0 (3) | 1.8 ± 0.2 (4)* | 3.0 ± 0.0 (2) |
| | 20°C | 4.3 ± 0.7 (5) | 3.5 ± 0.8 (4)* | 1.9 ± 0.1 (5)* | 1.2 ± 0.6 (5) |
| | 30°C | 2.2 ± 0.8 (5) | 1.5 ± 0.2 (4)* | 4.1 ± 2.0 (5) | 9.4 ± 2.0 (3) |

New Multiple Range Test was used to find significance of differences among temperature means. A preliminary analysis of variance showed no sex effect in either the constant or fluctuating salinity conditions, so sex was not included in any further ANOVA's. Snail hemolymph osmolality and ion concentrations were compared with seawater values by *t*-tests for each temperature-salinity combination. A significant difference ($P \leq 0.05$) would indicate regulation of hemolymph osmolality or ion concentrations as compared to seawater values.

### *Statistical analysis: Salinity fluctuation experiments*

Data from the salinity fluctuation experiments were also standardized by expressing hemolymph osmolality and ion concentrations as hemolymph minus seawater concentrations.

An ANOVA was run testing the effects of temperature, salinity regime, high versus low stage of fluctuation cycle, and time on percent tissue water, hemolymph osmolality, $Cl^-$, $Na^+$, $Mg^{2+}$, and $K^+$ concentrations of snails under fluctuating salinity regimes. For the ANOVAs time was broken into four classes: Days 1 and 3, 6 and 9, 12 and 15, and 18 and 21. This is a $3 \times 2 \times 2 \times 4$ factorial design. Duncan's New Multiple Range Test was used to detect significant differences ($P \leq 0.05$) among means of the dependent variables over time and to compare the three temperature experiments. Maximum R-square regressions of all dependent variables against time were performed for each temperature-salinity-regime stage-of-cycle combination. Regression lines are plotted as predicted values with standard errors. All statistical analyses were done using the Statistical Analysis System, Version 76.6 (Barr *et al.*, 1976).

## RESULTS

### *Constant salinity*

Temperature, salinity, and temperature-salinity interaction all had significant effects ($P \leq 0.05$) on the difference between hemolymph and seawater osmolality. Mean values for snails held at 7.5, 10, 20, and 30‰ salinity and 10°, 20°, and 30°C are given in Table I. Hemolymph osmolality increased as salinity increased. The *t*-test comparisons of hemolymph osmolality against seawater osmolality indicate a significant difference between hemolymph and seawater osmolality at 10, 20, and 30‰ and 10°C (Table I). There are statistically significant differences at two salinities under both the 20° and 30°C regimes, but the differences are smaller. Hemolymph osmolality was maintained slightly above seawater values at 10°C and was isosmotic or slightly hyperionic at 20° and 30°C. Percent tissue water was unaffected by temperature and decreased as ambient salinity and hemolymph osmolality increased (Table I).

Hemolymph concentrations of all ions studied increased as salinity increased under steady state conditions. Temperature, salinity, and temperature-salinity interactions all had significant effect ($P \leq 0.05$) on the difference between hemolymph and seawater $Cl^-$, $Na^+$, and $Mg^{2+}$ concentrations (Table I). Hemolymph $Cl^-$ concentrations were generally lower than seawater values. At 10°C hemolymph $Na^+$ concentrations were greater than seawater concentrations, while hemolymph $Na^+$ concentrations did not differ from ambient levels at 20° and 30°C. Hemolymph $[Mg^{2+}]$ was isionic or slightly hyperionic to ambient seawater at most temperature-salinity combinations examined. The *t*-test comparisons indi-

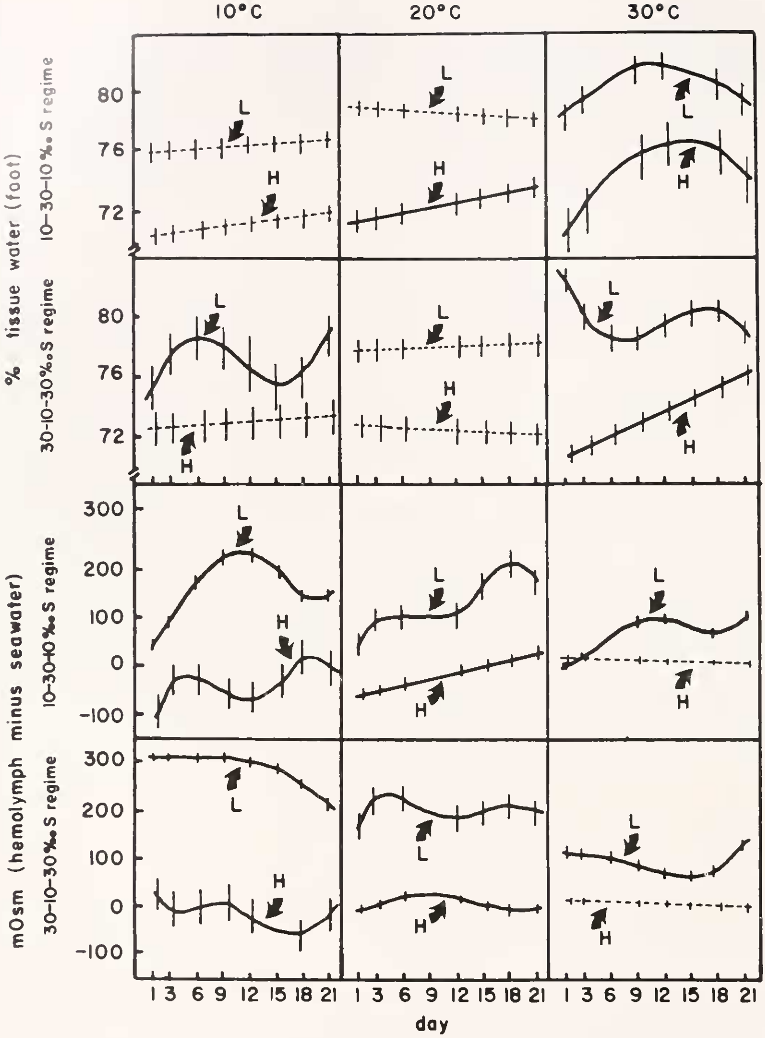

FIGURE 1. Regression lines for hemolymph osmolality of *Thais haemastoma* expressed as hemolymph osmolality minus seawater osmolality at the high (H) and low (L) phases of salinity cycles. Percent body water of the foot at high and low phases of salinity cycles are also given. Lines are presented for 10–30–10‰ and 30–10–30‰ S cycles at 10°, 20°, and 30°C. Vertical lines represent standard error at each sample point.

cated significant differences between hemolymph and seawater $Mg^{2+}$ concentrations at 7.5, 10, and 20‰ S at both 10° and 20°C. Although neither temperature nor salinity had significant effects on $K^+$ hemolymph-minus-seawater differences, the effect of temperature-salinity interactions was significant ($P \leq 0.05$). Hemolymph $[K^+]$ increased with increasing seawater concentration and was unaffected by temperature. The *t*-test comparisons for difference between seawater and hemolymph $[K^+]$ showed significant differences at 10 and 20‰ salinity.

### *Fluctuating salinity experiments*

Hemolymph osmolality tracked that of ambient seawater at all three temperatures under both salinity regimes. Hemolymph tended to be isosmotic or slightly hyposmotic during high salinity slackwater periods and to be hyperionic during low salinity slackwaters. This trend was most evident at 10°C. Figure 1 shows maximum R-square regression lines of hemolymph-minus-seawater osmolality for high and low salinity sample periods at 10°, 20°, and 30°C under both salinity regimes. Broken lines indicate nonsignificant, best-fit lines.

Analysis of variance (ANOVA) showed temperature, salinity regime, stage of cycle (high or low), time, and all two way interactions of these variables to have significant ($P \leq 0.05$) effects on the difference of osmolality between hemolymph and seawater. Duncan's New Multple Range Test showed that hemolymph-minus-seawater osmolality was greatest at 10°C, intermediate at 20°C, and least at 30°C over the 21-day salinity fluctuation experiment. Duncan's test also showed that hemolymph-minus-seawater osmolality was greater in snails under the 30–10–30‰ S fluctuating salinity regime than in snails under the 10–30–10‰ S regime. A large positive difference at low salinity slackwaters, as was observed at 10°C and to a lesser extent at 20° and 30°C (Fig. 1), indicates snail hemolymph was not closely tracking ambient levels during this phase of the salinity cycle.

Tissue water in the foot varied inversely with seawater and hemolymph osmolality, increasing with decreasing salinity (Fig. 1). ANOVA showed that temperature, stage of cycle, and time had significant effects ($P \leq 0.05$) on tissue water content. Tissue water was greatest at 30°C, intermediate at 20°C, and least at 10°C according to Duncan's test. This difference in tissue water content was obvious at the time of sampling. Bleeding of the snails was more difficult at 10°C than at the other temperatures and at 30°C the foot hemolymph was evident even after blotting the severed foot.

Temperature and stage of cycle, but not salinity regime, had significant ($P \leq 0.05$) effects of hemolymph $Cl^-$ values expressed as hemolymph-minus-seawater concentrations when salinity was fluctuated. All two way interactions, including those involving salinity regime, were significant. Duncan's New Multiple Range Test showed hemolymph-minus-seawater values to be greatest at 10°C and least at 30°C. Hemolymph-minus-seawater $[Cl^-]$ was greater than zero, indicating hemolymph $Cl^-$ was maintained above seawater values at most low-salinity sample points at 10° and 20°C (Fig. 2). At 30°C hemolymph-minus-seawater $Cl^-$ concentration was close to zero at both high and low salinity sample points, *i.e.*, hemolymph $Cl^-$ tracked ambient water concentrations closely at 30°C.

Somewhat similar trends were observed for hemolymph-minus-seawater $Na^+$ concentrations. Figure 2 shows that at 10°C hemolymph-minus-seawater $Na^+$ concentration was consistently greater than zero during the low-salinity sample periods while there was little difference between hemolymph and seawater concentrations at high-salinity sample points. The data were more variable at the higher temperatures but tend to show hemolymph $Na^+$ concentration tracking ambient levels more than at 10°C. ANOVA showed that temperature, salinity regime, stage of cycle, time, and all two way interactions involving these variables, except stage of cycle by time interaction, have significant effects on hemolymph-minus-seawater $[Na^+]$. Duncan's test showed hemolymph-minus-seawater $Na^+$ to be greatest at 10°C and least at 30°C and to be greater in snails under the 30–10–30‰ salinity regime (30‰ S acclimation) than in those under the 10–30–10‰ S regime.

Hemolymph $Mg^{2+}$ concentrations showed trends similar to those observed for the other ions studied. Hemolymph $[Mg^{2+}]$ was hyperionic during low-salinity sample periods and usually isionic or slightly hypoionic during high-salinity sample periods (Fig. 3). Temperature, stage of cycle, time, and all two way interactions

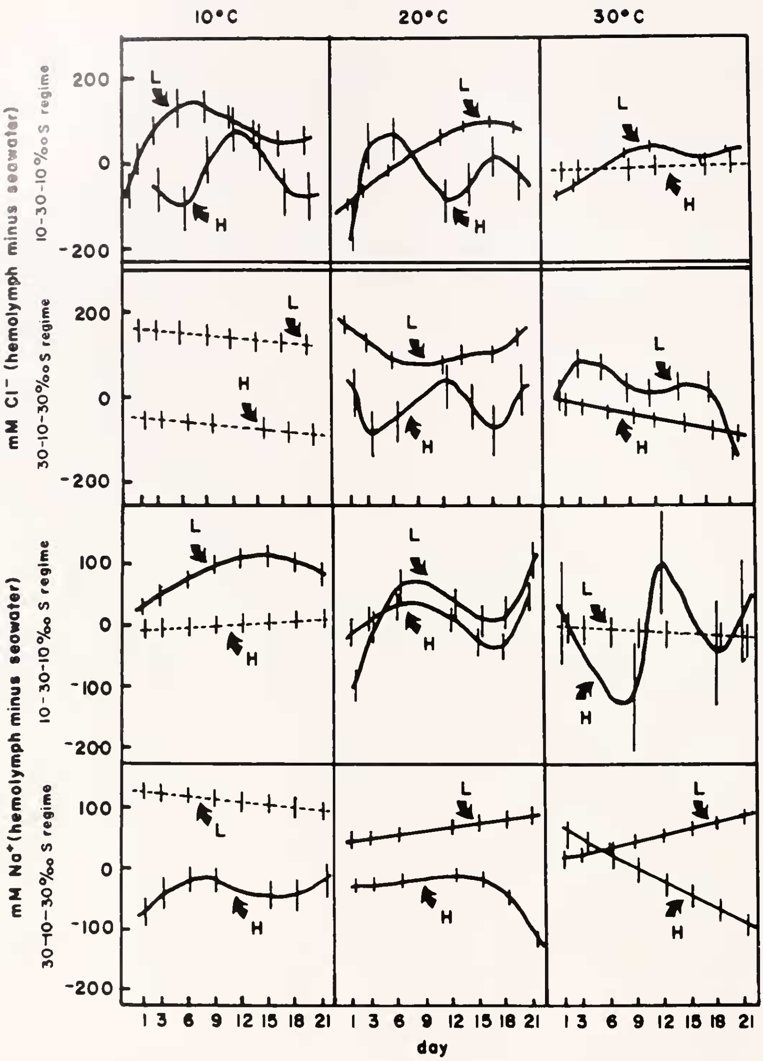

FIGURE 2. *Thais haemastoma.* Regression lines for hemolymph $Cl^-$ and $Na^+$ concentration of *Thais haemastoma* expressed as hemolymph-minus-seawater concentration at the high (H) and low (L) phases of salinity cycles. Lines are presented for 10–30–10‰ and 30–10–30‰ S cycles at 10°, 20°, and 30°C. Vertical lines represent standard error at each sample point.

tested except stage of cycle by time had significant effects ($P \leq 0.05$) on hemolymph-minus-seawater $Mg^{2+}$. Duncan's test showed that hemolymph-minus-seawater [$Mg^{2+}$] was greater at 10°C than at 20° and 30°C.

Hemolymph [$K^+$] also tended to be isionic to ambient seawater during high-salinity sample periods and to be hyperionic at low-salinity sample points (Fig. 3). Hemolymph $K^+$ was less hyperionic at low-salinity sample points at 30°C than at the other temperatures, indicating it tracked ambient levels to a greater extent. Temperature, salinity regime, and stage of cycle all significantly affected ($P \leq 0.05$) hemolymph $K^+$ expressed as hemolymph-minus-seawater concentration,

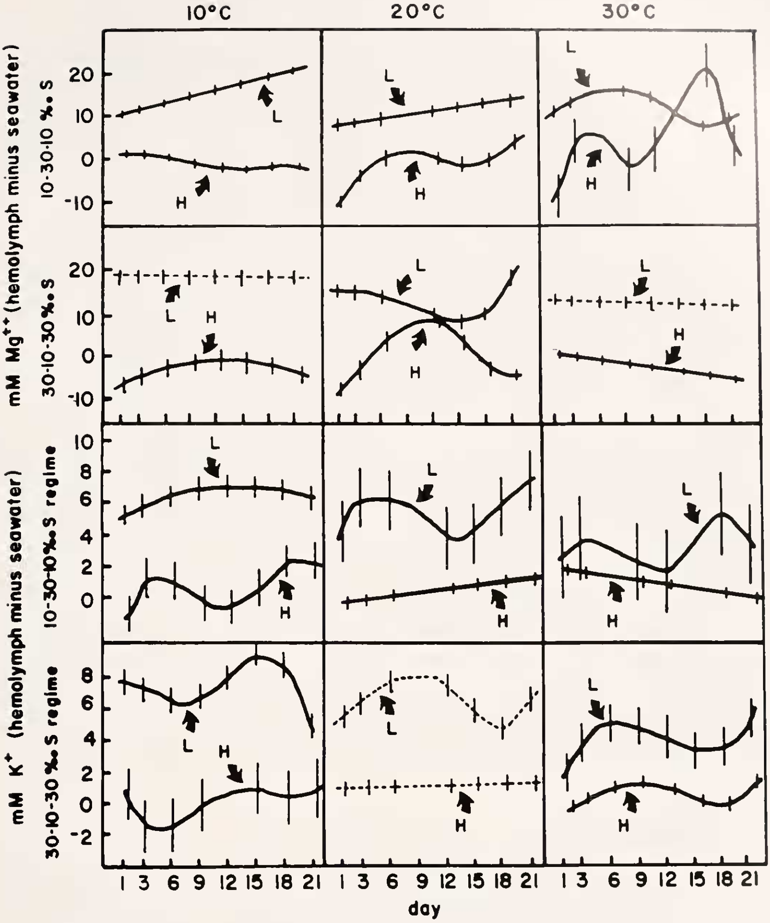

FIGURE 3. Regression lines for hemolymph Mg$^{2+}$ and K$^{+}$ concentrations of *Thais haemastoma* expressed as hemolymph-minus-seawater concentration at the high (H) and low (L) phases of salinity cycles. Lines are presented for 10–30–10‰ and 30–10–30‰ S cycles at 10°, 20°, and 30°C. Vertical lines represent standard error at each sample point.

although no interactions were significant. Duncan's test showed hemolymph minus seawater [K$^{+}$] was greatest at 10°C and least at 30°C.

### *Ninhydrin-positive substances*

Ninhydrin-positive-substances (NPS) concentration of the foot, calculated as $\mu$M NPS/g dry weight and as $\mu$M NPS/g tissue water, are presented in Table II. Data are shown for 15 steady-state temperature-salinity combinations. NPS content of the foot expressed as either NPS/g dry weight or NPS/g tissue water increases with increasing salinity at all three temperatures studied. NPS content of the foot tends to be lower at 10°C than at the other temperatures. No other clear trends are evident.

NPS/g dry weights were not significantly different from control (day 0) values at high- and low-salinity sample points on the first day of salinity fluctuations (Fig. 4). Snails acclimated to 30‰ S had higher NPS levels in the foot even at

TABLE II

*Ninhydrin-positive substances (NPS) in the foot of* Thais haemastoma *acclimated to 15 temperature-salinity combinations expressed as both μM NPS/g dry weight and as μM NPS/g tissue water. Values given are* $\bar{X}$ ± *95% confidence limit (n). ND indicates no data available.*

| | 7.5‰ | 10‰ | 20‰ | 30‰ | 35‰ |
|---|---|---|---|---|---|
| 10°C | | | | | |
| μM/g dry weight | 87.0 ± 19.4 (6) | 144.7 ± 3.3 (5) | 183.6 ± 10.5 (4) | 268.2 ± 31.6 (4) | ND |
| μM/g tissue $H_2O$ | 23.6 ± 4.0 (6) | 40.9 ± 8.0 (4) | 94.3 ± 7.6 (4) | 94.3 ± 7.6 (4) | ND |
| 20°C | | | | | |
| μM/g dry weight | 124.3 ± 1.7 (5) | 129.0 ± 14.5 (5) | 211.0 ± 7.3 (5) | 312.0 ± 34.5 (4) | 338.1 ± 25.3 (1) |
| μM/g tissue $H_2O$ | 37.3 ± 2.2 (5) | 41.0 ± 5.2 (5) | 66.8 ± 9.4 (4) | 96.5 ± 18.7 (4) | 117.37 ± 10.4 (4) |
| 30°C | | | | | |
| μM/g dry weight | 115.4 ± 3.4 (4) | 159.9 ± 9.9 (5) | 217.64 ± 45.4 (3) | 244.6 ± 51.3 (3) | 241.19 ± 24.7 (3) |
| μM/g tissue $H_2O$ | 34.4 ± 3.2 (4) | 48.7 ± 4.5 (4) | 68.1 ± 4.2 (3) | 93.6 ± 5.8 (3) | 84.9 ± 2.0 (3) |

corresponding points of the salinity fluctuation cycle, that is, when both were measured at 10 or 30‰ S. NPS/g dry weight of snails acclimated to 30‰ and subjected to salinity cycles of 30–10–30‰ S did not change significantly over the 3-week experiment at 10°, 20°, or 30°C. In contrast, snails acclimated to 10‰ S and subjected to salinity cycles of 10–30–10‰ exhibited significantly higher NPS/g dry weight values than control and day 1 values after 2 weeks and remained higher after 3 weeks of cycling salinity. There was little significant cycling of NPS levels, expressed as μM NPS/g dry weight, with the daily salinity fluctuations. Similar trends were observed when these data were analyzed expressing NPS levels as μM NPS/g tissue water except that NPS levels cycled with fluctuating salinity to a greater extent, tending to be higher during high salinity slackwater sample periods (Fig. 4).

## DISCUSSION

Although little difference existed between hemolymph and seawater osmolality or ion concentrations in *Thais haemastoma* at constant salinities and temperatures studied, temperature affected hemolymph values over a range of salinities. The differences between hemolymph and seawater osmolality and $Cl^-$ and $Na^+$ concentrations were greatest at 10°C. $Mg^{2+}$ hemolymph-minus-seawater concentration was less at 10° and 30°C than at 20°C, while hemolymph $K^+$ levels were not affected by temperature under steady-state conditions. Pierce (1970) argued that the slight hyperosmotic concentrations of blood and pericardial fluids relative to sea water in *Modiolus* was due to a Gibbs-Donnan equilibrium. However, Mangum and Johansen (1975) have shown that a Gibbs-Donnan equilibrium is not responsible for the frequently observed hyperosmoticity of body fluids relative to ambient water in osmoconforming marine invertebrates. Therefore, mechanisms responsible for *T. haemastoma* maintenance of hemolymph osmolality, $Na^+$, $Mg^{2+}$, $K^+$, and $Cl^-$ levels at concentrations significantly different from that of the ambient water remain to be elucidated.

When salinity was cycled, hemolymph osomolality and ion concentrations were maintained above seawater values during the low phase of salinity cycles and were usually isosmotic during the high phase at 10°, 20°, and 30°C. Less cycling of hemolymph ion concentrations with salinity cycles occurred at 10°C than at 30°C, especially of hemolymph $Mg^{2+}$ and $K^+$ concentrations, but there were still significant hemolymph changes during most cycles. Similar results have been reported for

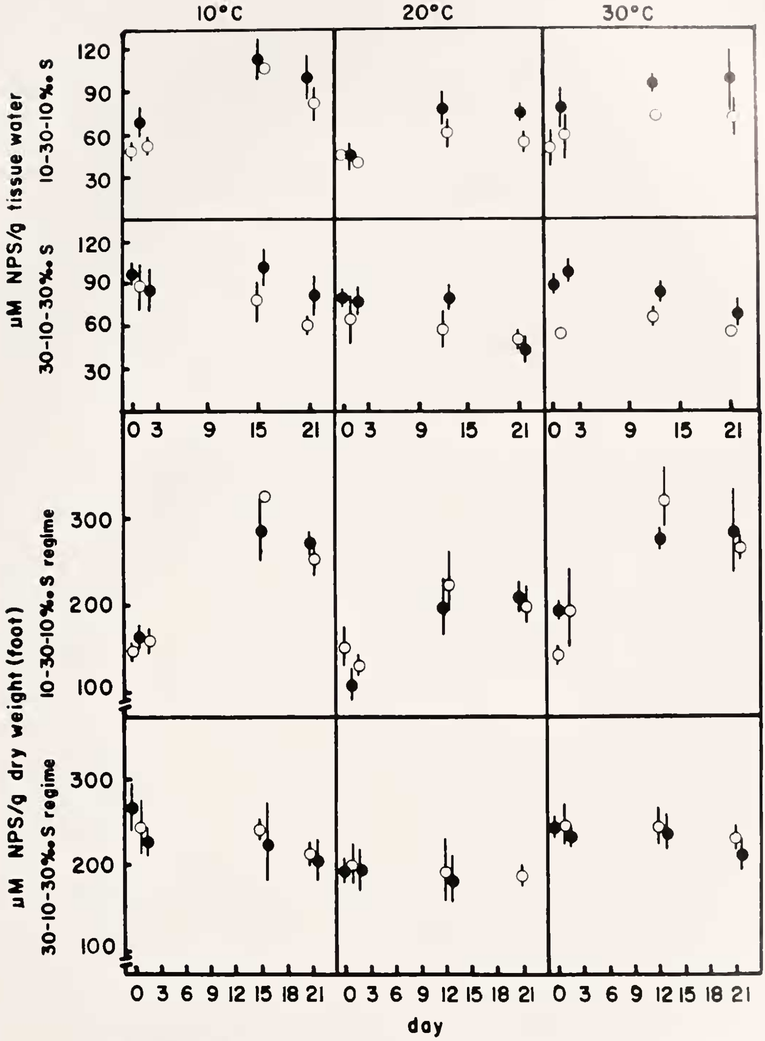

FIGURE 4. *Thais haemastoma.* Mean plus or minus the 95% confidence limits are given for NPS/g dry weight and NPS/g tissue water in the foot of *Thais haemastoma* at the high and low phase of salinity cycles. Data are given for 10–30–10‰ and 30–10–30‰ S cycles at 10°, 20°, and 30°C. Open circles represent high salinity sample points; closed circles, low salinity points. Lack of error bars indicates confidence limits smaller than the point on the figure.

*T. haemastoma* exposed to 14 days of a 20–10–20‰ S diurnal pattern of fluctuating salinity at 15°–21°C. (Stickle and Howey, 1975).

The observed hemolymph-to-seawater ion differences during salinity fluctuation in *Thais haemastoma* are probably passive. Hemolymph was isionic during the high phase of salinity cycles and hyperionic during the low phase, suggesting greater solute and solvent movement during periods of increasing salinity. Extracellular fluid of other osmoconforming molluscs (Stickle and Ahokas, 1975; Stickle and Howey, 1975; Hand and Stickle, 1977, Shumway, 1977) and echinoderms (Stickle and Ahokas, 1974) may also remain hypersomotic during the low phase of salinity cycles. If the hemolymph-to-seawater difference in ion concentration during the low phase of salinity cycles at 10°C were caused by metabolic-dependent activities, regulation would have been observed under steady-state conditions also. Magnesium was the only hemolymph ion maintained at levels significantly different from seawater values under steady-state conditions. All other hemolymph ions were not different from seawater values even after 5 weeks acclimation to 10°C, suggesting low-temperature changes in membrane permeability or changes in other passive activities during salinity fluctuations. Prusch and Hall (1978) found that *Mytilus edulis* is capable of altering tissue water permeability with changes in osmolality of the ambient water. Similar permeability changes may be occurring in response to temperature and fluctuating salinity in *T. haemastoma*. Oxygen consumption in *T. haemastoma* exposed to single salinity cycles at 20°C decreases in the middle of 10–15–10‰, 30–10–30‰ and 10–30–10‰ S diurnal cycles (Findley *et al.*, 1978), suggesting a passive mechanism is responsible for the differences between hemolymph and seawater during the low phase of salinity cycles.

*Thais haemastoma* maintains higher hemolymph osmolality at lower temperatures under both steady-state and fluctuating salinity conditions. Hemolymph osmolality and ion concentrations are regulated above seawater values during the low phase of salinity cycles to a much greater extent at 10°C than at 30°C. Thus it seems *T. haemastoma* is better able to withstand dilute and widely fluctuating salinities at low temperatures. Lowest field salinities in Barataria Bay, the collection site for experimental animals, occur during winter (Hewatt, 1951). *T. haemastoma* may be able to compensate for low environmental salinities by passively regulating hemolymph composition when natural tidal salinity fluctuations occur most frequently. Since the feeding threshold of *T. haemastoma* is about 12.5°C (Garton and Stickle, 1980) it cannot be concluded that *T. haemastoma* would be able to permanently colonize dilute waters at cold temperatures. Indeed it is generally considered to be a warm water species extending only as far north as North Carolina.

Ninhydrin-positive substances (NPS) increased in foot tissue of *T. haemastoma* with increasing salinity and were lower at 10°C than at other experimental temperatures under steady-state salinity conditions. Ninhydrin-positive substances are principally composed of free amino acids which are important in cell volume regulation in response to salinity variation. Peterson and Duerr (1969) found the free amino acid levels of *Tegula funebralis* maintained for 11 days at 50, 100, 120, and 160% seawater (35‰ S = 100%) to increase linearly from 50 to 120% seawater and then to decline to approximately 100% seawater concentration at 160% seawater when based on grams/wet-weight. *Modiolus* hearts (Pierce and Greenberg, 1973) and intact *Mytilus edulis* (Livingstone *et al.*, 1979) transferred directly from high to low salinity release free amino acids from the intracellular fluid compartment. Free amino acids increase in acclimation of bivalve molluscs to high salinity (Baginski and Pierce, 1977, Gainey, 1978). Expressing NPS concentration in the foot of *T. haemastoma* on a dry weight basis indicates synthesis or degradation of NPS, principally free amino acids, whereas expressing NPS concentration on a tissue water basis provides information on the osmotic effectiveness of free amino acids. Extracellular fluid concentrations of NPS are small in comparison with entire foot NPS, suggesting that most NPS are intracellular. Stickle and Howey (1975) found hemolymph NPS concentrations to range from 0.6 to 6.4 mM in snails acclimated to 10–30‰ S. Foot tissue water NPS concentrations range from 41 to 117 mM between 10 and 30‰ S.

Variation in the volume of the intracellular fluid compartment without a concomitant change in absolute NPS content will alter the osmotic importance of NPS. Staaland (1970) found the intracellular fluid compartment of *Buccinum undatum* to vary inversely with salinity between 10 and 33‰ whereas no change occurred in the size of the extracellular fluid compartment. If a similar relationship exists in the foot of *T. haemastoma* the osmotic effectiveness of NPS as intracellular osmotically active particles will be further reduced by dilution of the intracellular fluid compartment at low salinity.

Under fluctuating salinity conditions, it was more difficult to obtain hemolymph at 10°C than at 20°C while hemolymph was most abundant at 30°C. Stickle and Howey (1975) found similar results with *T. haemastoma* exposed to two weeks of diurnal 20–10–20‰ patterns of fluctuating salinity. Total body water in the foot of stepwise acclimated *T. haemastoma* was not significantly different between 10° and 30°C, which suggests that the intracellular fluid compartment was probably larger at 10°C than at 20° or 30°C.

Ninhydrin positive substances did not cycle in the foot of *T. haemastoma* in response to cycling salinities, although NPS levels increased with time in snails acclimated to 10‰ S and subjected to a long-term 10–30–10‰ regime. In contrast, all eight bivalve species studied by Shumway *et al.* (1977) cycled adductor muscle NPS levels in response to 1-day salinity fluctuations. After 1 week of salinity fluctuation, no changes in *Mytilus edulis* tissue NPS levels were observed during a salinity cycle. Additionally, tissue NPS did not decline below the concentration observed in 100% seawater. On the other hand, Livingstone *et al.*, (1979) found changes in *M. edulis* NPS level to be small during short-term salinity fluctuations (30–15–30‰ cycles) but to decline markedly after exposure to 24 days of salinity fluctuations. These authors believe that the lack of NPS decline during fluctuating salinity observed in the study of Shumway *et al.*, (1977) may have been due to a combination of valve closure during the low-salinity phase of each cycle and salinity effects. The mussels used by Livingstone *et al.*, (1979) remained open throughout salinity cycles and continued to respire. Likewise, the *T. haemastoma* used in our fluctuating-salinity experiments remained attached to aquarium walls and crawled and preyed on oysters, although less activity was observed at 10°C than at other temperatures studied. Garton and Stickle (1980) have documented the feeding activity of oyster drills on oysters under identical conditions and Findley *et al.*, (1978) have shown aerobic respiration to occur under fluctuating salinity conditions.

*Thais haemastoma* passively regulates hemolymph osmolality and ionic composition slightly above environmental levels at low temperatures and constant salinity, as well as during the daily low phase of long-term salinity fluctuation cycles. Since cell fluid volume appears to be greater at low temperatures and low salinities, NPS may be less important in osmotic regulation and cell volume regulation under conditions of low environmental temperature and salinity.

This research was partially supported by a PRECOL Research Fellowship to J.E.H. during the summer and was done as partial fulfillment of the requirements for the Master of Science Degree. Tom Sabourin, Dave Garton, Preston Newman and Barbara Belisle provided technical assistance during this project. The authors wish to acknowledge with thanks the critical review of this manuscript offered by Dr. Thomas Dietz, Dr. John Fleeger, Dr. Earl Weidner, and Mr. David Saintsing.

## SUMMARY

1. When *Thais haemastoma* were acclimated stepwise to constant salinity; hemolymph osmolality, $Na^+$, $Mg^{2+}$, $K^+$, and $Cl^-$ concentrations as well as tissue NPS concentration increased with salinity between 7.5 and 30‰ at 10°, 20°, and 30°C. Hemolymph osmolality was significantly greater than that of seawater at 10, 20, 30‰ S and 10°C. Per cent tissue water was unaffected by temperature and decreased with increasing salinity and hemolymph osmolality. The hemolymph concentration of all ions increased with increasing concentrations in seawater. Hemolymph $[Cl^-]$ was lower than in seawater at 20° and 30°C but hyperionic to seawater at 20 and 30‰ S and 10°C. Hemolymph $[Na^+]$ was greater than in seawater at 10°C but no difference existed at 20° and 30°C. Hemolymph $[Mg^{2+}]$ was significantly greater than in seawater at 10 and 20‰ S at all temperatures. Neither temperature nor salinity significantly affected the hemolymph-to-seawater difference in $[K^+]$. [NPS] in the foot of *T. haemastoma* increased with salinity and was lower at 10°C than at 20° and 30°C.

2. Hemolymph osmolality tracked seawater osmolality less well at 10°C than at 20° and 30° during 30–10–30‰ and 10–30–10‰ diurnal patterns of fluctuating salinity. Reduced tracking at 10°C was due to less decline of hemolymph osmolality during the decreasing-salinity phase of the cycles. Snail hemolymph osmolality also declined less during the decreasing-salinity phase of the 30–10–30‰ S cycle than during the 10–30–10‰ S cycles, indicating that acclimation to different constant salinities prior to the initiation of fluctuating salinity cycles has a significant effect on the response of *T. haemastoma* to fluctuating salinity. Tissue water in the foot varied inversely with seawater and hemolymph osmolality during fluctuating salinity. The difference in percent tissue water between snails acclimated to 10 and 30‰ S prior to salinity fluctuation was greatest at 30°C, intermediate at 20°C, and least at 10°C. Hemolymph $[Na^+]$, $[Mg^{2+}]$, $[K^+]$, and $[Cl^-]$, tracked ambient salinity least well at 10°C and most closely at 30°C. Passive factors are likely to be responsible for the differences in hemolymph osmotic and ionic fluctuation observed at 10°, 20°, and 30°C. Tissue NPS did not fluctuate with daily salinity cycles in either the 30–10–30‰ or 10–30–10‰ diurnal patterns of fluctuating salinity. Foot tissue NPS of snails acclimated to 30‰ did not change over three weeks of exposure to a 30–10–30‰ S pattern of fluctuating salinity but NPS in the foot of snails acclimated to 10‰ S and subjected to a 10–30–10‰ S pattern of fluctuating salinity increased significantly in 15 days and remained significantly higher on Day 21.

## LITERATURE CITED

BAGINSKI, R. A., AND S. K. PIERCE, 1977. The time course of intracellular free amino acid accumulation in tissues of *Modiolus demissus* during high salinity adaptaton. *Comp. Biochem. Physiol.*, **57**: 407–412.

BARR, A. J., J. H. GOODKNIGHT, J. P. SALL, AND J. T. HELWIG, 1976. *A User's Guide to SAS 76.* Raleigh, North Carolina: SAS Institute Inc.

DEHNEL, P. A., 1962. Aspects of osmoregulation in two species of intertidal crabs. *Biol. Bull.*, **122**: 208–227.

DEHNEL, P. A., AND T. H. CAREFOOT, 1965. Ion regulation in two species of intertidal crabs. *Comp. Biochem. Physiol.*, **15**: 377–397.

Findley, A. M., B. W. Belisle, and W. B. Stickle, 1978. Effects of salinity fluctuations on the respiration rate of the Southern Oyster Drill *Thais haemastoma* and the Blue Crab *Callinectes sapidus*. *Mar. Biol.*, **49**: 59–67.

Gainey, L. F., Jr., 1978. The response of the Corbiculidae (Mollusca: Bivalvia) to osmotic stress: the cellular response. *Physiol. Zool.*, **51**: 79–91.

Garton, D., and W. B. Stickle, 1980. Effects of salinity and temperature on the predation rate of *Thais haemastoma* on *Crassostrea virginica* spat. *Biol. Bull.*, **158**: 49–57.

Hand, S. C., and W. B. Stickle, 1977. Effects of tidal fluctuations of salinity on the pericardial fluid composition of the American Oyster *Crassostrea virginica*. *Mar. Biol.*, **33**: 309–322.

Hewatt, W. G., 1951. *Salinity studies in Louisiana coastal embayments west of the Mississippi River*. 32 pp. Final Report of Project Nine. Texas A & M. Research Foundation College Station, Texas.

Kinne, O., 1970. Temperature: Invertebrates. Pp. 407–514 in O. Kinne, Ed., *Marine Ecology, Vol. 1. Environmental Factors, Part 1.* London: Wiley-Interscience.

Kinne, O., 1971. Salinity: Invertebrates. Pp. 821–996 in O. Kinne, Ed., *Marine Ecology Vol. 1. Environmental Factors, Part II.* London: Wiley-Interscience.

Livingstone, D. R., J. Widdows, and P. Fieth, 1979. Aspects of nitrogen metabolism of the common mussel *Mytilus edulis:* adaptation to abrupt and fluctuating changes in salinity. *Mar. Biol.*, **53**: 41–56.

Lockwood, A. P. M., 1960. Some effects of temperature and salinity of the medium on the ionic regulation of the isopod *Asellus aquaticus* (L.). *J. Exp. Biol.*, **37**: 614–630.

Mangum, C. P., and K. Johansen, 1975. The colloid osmotic pressures of invertebrate body fluids. *J. Exp. Biol.*, **63**: 661–671.

Peterson, M. B., and T. G. Duerr, 1969. Studies on osmotic adjustment in *Tegula funebralis* (Adams, 1954). *Comp. Biochem. Physiol.*, **28**: 633–644.

Pierce, S. K., Jr., 1970. The water balance of *Modiolus* (Mollusca: Bivalvia): osmotic concentrations in changing salinities. *Comp. Biochem. Physiol.*, **36**: 521–533.

Pierce, S. K., and M. J. Greenberg, 1973. The initiation and control of free amino acid regulation of cell volume in salinity stressed marine bivalves. *J. Exp. Biol.*, **59**: 435–440.

Prusch, R. D., and C. Hall, 1978. Diffusional water permeability in selected marine bivalves. *Biol. Bull.*, **154**: 292–301.

Rosen, H., 1957. A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.*, **67**: 10–15.

Shumway, S. E., 1977. The effect of fluctuating salinity on the tissue water content of eight species of bivalve molluscs. *J. Comp. Physiol.*, **116**: 269–285.

Shumway, S. E., P. A. Gabbot, and A. Youngson, 1977. The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin positive substances in the adductor muscles of eight species of bivalve molluscs. *J. Exp. Mar. Biol. Ecol.*, **29**: 131–151.

Staaland, H., 1970. Volume regulation in the common whelk, *Buccimun undatum* L. *Comp. Biochem. Physiol.*, **34**: 355–365.

Stickle, W. B., and R. Ahokas, 1974. The effects of tidal fluctuation of salinity on the pericardial fluid composition of several echinoderms. *Comp. Biochem. Physiol.*, **47**: 469–476.

Stickle, W. B., and R. Ahokas, 1975. The effects of tidal fluctuation of salinity on the hemolymph composition of several molluscs. *Comp. Biochem. Physiol.*, **50**: 291–296.

Stickle, W. B., and T. W. Howey, 1975. Effects of tidal fluctuations of salinity on hemolymph composition of the Southern Oyster Drill *Thais haemastoma*. *Mar. Biol.*, **33**: 309–322.

Taylor, E. W., P. J. Butler, and A. Al-Wassia, 1977. The effect of a decrease in salinity on respiration, osmoregulation, and activity in the shore crab, *Carcinus maenus* (L.) at different acclimation temperatures. *J. Comp. Physiol.*, **119**: 155–170.

Todd, M. E., 1963. Osmoregulation in *Ligea oceanica* and *Iodotea granulosa*. *J. Exp. Biol.*, **40**: 381–392.

Verway, J., 1957. A plea for the study of temperature influence on osmotic regulation. *Annee Biol. 3 serie*, **33**: 129–149.